Cell Stem Cell
Previews

Human Somatic Cell Reprogramming: Does the Egg Know Best?

Alan Colman[1,2,*] and Justine Burley[3]
[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[2]Genea Biocells Pty Limited, Sydney, NSW 2000, Australia
[3]Gaibian Associates, 257 River Valley Road, Singapore 138638
*Correspondence: colman@fas.harvard.edu
http://dx.doi.org/10.1016/j.stem.2014.10.011

Somatic cell nuclear transfer offers an alternative approach to the use of exogenous transcription factors for the reprogramming of somatic cells. But is it a better way? Two groups have performed detailed molecular comparisons between human cell lines made by the two methods and report different conclusions.

Mouse embryonic stem cells (mESCs) and induced pluripotent stem cells (miPSCs) can, in the right circumstances, form any cell type of the adult body. Their human counterparts, hESCs and hiPSCs, probably possess similar pluripotential attributes although the range of tests available to confirm this is circumscribed by obvious ethical constraints. Both of these human cell types have been feted for their potential to provide new, in vitro disease modeling modalities and replacement tissues for sick human patients. hiPSCs are considered by many to be superior to hESCs because autologous lines, as well as a broad range of disease-specific lines, can be prepared in an ethically uncontentious manner. However, niggling questions remain as to their functional and molecular similarity to hESCs, a debate muddied by the genetic diversity of the pluripotent lines being compared. Unfortunately, in vitro fertilization-derived hESCs (IVF-hESCs) and hiPSC lines containing the same donor genome (isogenic) are simply not available for comparison. However, recent success in reprogramming somatic nuclei by somatic cell nuclear transfer (SCNT) (Tachibana et al., 2013) has allowed comparisons to be made between isogenic NT-hESC and hiPSC lines using nonrelated IVF-hESC as a convenient reference point.

In July this year, Ma et al. (2014) reported that NT-hESCs made from fetal fibroblast nuclei had transcriptional and DNA methylation signatures much closer to IVF-hESCs than did hiPSCs generated from the same fibroblasts, leading the authors to advocate the use of NT-hESCs over hiPSCs. Similar findings have been reported for mouse NT-ESCs and iPSCs (Liang and Zhang 2013). In this issue of *Cell Stem Cell*, Johannesson et al. (2014) employ similar and new tests to examine isogenic NT-hESC and hiPSC lines made from neonatal and adult cell sources. Like Ma et al. (2014), they note that both cell types display genetic and epigenetic changes not seen in the somatic donor populations. However, in contrast to Ma et al. (2014), they find the scale and nature of the epigenetic changes similar for both cell types and conclude that reprogramming per se, rather than the exact method used, is responsible. This is puzzling.

Both groups each analyzed two sets of isogenic NT-hESC and hiPSC lines alongside genetically unrelated IVF-hESC and fibroblast somatic donor cells. Ma et al. (2014) looked first for structural genome changes and found on average 1.8, 0.8, and 0.5 de novo copy number variations (CNVs) in early-passage hiPSCs, NT-hESCs, and IVF-hESCs, respectively. These differences were not statistically significant. Johannesson et al. (2014) focused on nonstructural, coding mutations and determined on average 10.57 and 10.43 de novo mutations in seven NT-hESC and seven hiPSC lines derived from neonatal foreskin and adult dermal fibroblast somatic donors, respectively. Again, these differences were not significant. Most of the mutations were found in lines derived from adult donors and many were shared between independently isolated lines, indicating they were in the initial parental population as shown previously (Gore et al., 2011). When these data were augmented with analysis from triploid NT-hESCs (Noggle et al., 2011) and parthenogenetically generated hESC lines, the authors concluded that most mutations pre-existed in the donor cells or arose during reprogramming rather than in postreprogramming culture. However, the concordance seen above between the groups did not extend to the epigenetic changes observed.

Differentiation proceeds by the establishment of epigenetic regulation of the genome and involves covalent DNA modification, particularly DNA methylation. The methylation patterns of somatic and embryonic genomes are clearly distinct and these differences, inter alia, contribute to the diagnostic transcriptomes of every cell type and also underlie imprinting phenomena where genes or chromosomes (e.g., X inactivation) inherited from one of the parents are differentially expressed in all fetal and adult cells. Complete reprogramming demands a resetting of the epigenetic landscape and there have been many reports that factor-mediated reprogramming is aberrant and incomplete and that iPSC lines often retain an epigenetic memory of their previous somatic state (Kim et al., 2010; Liang and Zhang, 2013). Arguably, SCNT mimics human physiology more faithfully because it emulates normal fertilization where the sperm genome has to be radically restructured; it is certainly faster. Global DNA methylation patterns, transcriptomes, and parental and X chromosome imprinting were therefore examined by both groups. Ma et al. (2014) found that the DNA methylation patterns of NT-hESCs corresponded closely to those of IVF-hESCs while hiPSC patterns differed and retained 8-fold more sites indicative

of retained memory and 235-fold more sites reflective of random reprogramming errors. In contrast, Johannesson et al. (2014) found very little difference between the global methylation patterns of hiPSCs, NT-hESCs, and IVF-hESCs. Transcriptome analysis performed by both groups using RNA sequencing showed the same trends as their global methylation data, which is reassuring because methylation differences do not always lead to transcriptional differences (Ma et al., 2014). Finally, DNA methylation and transcriptional analysis and the status of imprinted loci, including X chromosomal loci, were compared across the different pluripotent cell lines. While both groups found that aberrant sites were more frequent in the NT-hESC and hiPSC lines, Ma et al. (2014) again found the NT-hESCs to have far fewer changes than hiPSCs.

Setting aside limitations to interpretation posed by the small sample sizes used, there are troublesome differences between the two sets of data reported above. Johannesson et al. (2014) flag two potential causes: different somatic cell donors (fetal versus neonatal and adult) and different methods of factor-mediated reprogramming (Sendai and retrovirus versus mRNA). Regarding somatic donors, intuitively one expects fetal donor nuclei to be more and not less sensitive to exogenous stimuli than the older sources. As for the reprogramming vectors, the early reports of mRNA-mediated reprogramming showed a much greater concordance of transcriptional expression between IVF-hESCs and the mRNA-hiPSCs than between IVF-hESCs and retroviral hiPSCs (Warren et al., 2010). If this proves to be the correct explanation, then further analysis of SCNT-mediated reprogramming may yield more insight into the speed and efficiency of reprogramming than into its quality (Shinagawa et al., 2014). Of course, irrespective of which explanation, if any, could reconcile the results of the two studies, neither paper addresses the issue of *functional* similarity between NT-hESCs and hiPSCs. This, as well as safety issues (concerning therapeutic uses), is ultimately what will determine whether a reprogrammed line is "fit for purpose." It is unclear whether reported genome-wide transcriptional differences between the various lines or epigenetic memory in newly reprogrammed lines preclude their usefulness for two reasons. The impact of changes in transcriptional patterns on the predisposition of pluripotent stem cell lines to form particular lineages is poorly understood and can be surprisingly specific (Kim et al., 2011). Also, epigenetic memory can be lost by extended culture times (Liang and Zhang, 2013). Doubtless, functional readouts will constitute the next phase of these groundbreaking studies and the results will be awaited with great interest.

REFERENCES

Gore, A., Li, Z., Fung, H.L., Young, J.E., Agarwal, S., Antosiewicz-Bourget, J., Canto, I., Giorgetti, A., Israel, M.A., Kiskinis, E., et al. (2011). Nature *471*, 63–67.

Johannesson, B., Sagi, I., Gore, A., Paull, D., Yamada, M., Golav-Lev, T., Li, Z., LeDuc, C., Shen, Y., Stern, S., et al. (2014). Cell Stem Cell *15*, this issue, 634–642.

Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., et al. (2010). Nature *467*, 285–290.

Kim, H., Lee, G., Ganat, Y., Papapetrou, E.P., Lipchina, I., Socci, N.D., Sadelain, M., and Studer, L. (2011). Cell Stem Cell *8*, 695–706.

Liang, G., and Zhang, Y. (2013). Cell Stem Cell *13*, 149–159.

Ma, H., Morey, R., O'Neil, R.C., He, Y., Daughtry, B., Schultz, M.D., Hariharan, M., Nery, J.R., Castanon, R., Sabatini, K., et al. (2014). Nature *511*, 177–183.

Noggle, S., Fung, H.L., Gore, A., Martinez, H., Satriani, K.C., Prosser, R., Oum, K., Paull, D., Druckenmiller, S., Freeby, M., et al. (2011). Nature *478*, 70–75.

Shinagawa, T., Takagi, T., Tsukamoto, D., Tomaru, C., Huynh, L.-M., Sivaraman, P., Kumarevel, T., Inoue, K., Nakato, R., Katou, Y., et al. (2014). Cell Stem Cell *14*, 217–227.

Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H.S., Sritanaudomchai, H., et al. (2013). Cell *153*, 1228–1238.

Warren, L., Manos, P.D., Ahfeldt, T., Loh, Y.H., Li, H., Lau, F., Ebina, W., Mandal, P.K., Smith, Z.D., Meissner, A., et al. (2010). Cell Stem Cell *7*, 618–630.